Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia

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Abstract

The biological consequences of mating interactions between indigenous and exotic biotypes of *Bemisia tabaci* (Gennadius) in Australia were studied using a combination of field and laboratory experiments. The key results of the interaction between the B and eastern Australian biotypes were reduced population increase, a marked increase in the proportion of male progeny, fewer eggs produced by females paired with males of different biotype and no difference in the numbers of eggs per unmated female and females paired with males of the same biotype. In addition, there was no change in the proportion of eggs hatching, mixed biotype pairs spent more time courting than single biotype pairs and a low level of hybridization in field cages and small containers was observed. These observations suggest three possibilities. The first is the 'distracting male hypothesis' in which mating pairs made up of different biotypes apportion more time to courtship and less time to egg laying than single biotype pairs. The second invokes the 'single-locus complementary sex determination model' in which the production of non-viable diploid male zygotes may explain the reduction in eggs laid. The third is cytoplasmic incompatibility between biotypes caused by *Wolbachia*. The results also suggest that the geographical distribution of clusters of related biotypes both overseas and in Australia may be explained by between-biotype interactions leading to the formation of parapatric populations.

Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is globally distributed, being found on all continents except Antarctica. It is haplo-diploid, producing males from unfertilized eggs. There are numerous different populations that, while morphologically indistinguishable (Rosell *et al*., 1997), vary considerably for many ecological, physiological and genetic factors (e.g. Costa & Brown, 1991; Liu *et al*., 1992; Bedford *et al*., 1994; Wool *et al*., 1994; Brown *et al*., 1995b; Costa *et al*., 1995; Wang & Tsai, 1996; De Barro & Driver, 1997; Frohlich *et al*., 1999; De Barro *et al*., in press). These factors have been used to characterize different world *B. tabaci* populations into a number of distinct biotypes (Bedford *et al*., 1994). At the chromosomal level there are no apparent differences between the biotypes compared in either chromosome structure or number (Blackman & Cahill, 1998).

Whether these differences are sufficient to warrant a taxonomic revision of *B. tabaci* is open to debate. The concept of what constitutes a species has been well summarized in Avise (1994) and Claridge *et al*. (1997) and, contrary to popular belief, the requirement for reproductive isolation is not necessary as interspecific mating can occur with production of fertile hybrids. However, all the discussion on whether *B. tabaci* is a species complex is based on the 'biological species concept' (Dobzhansky, 1937) which requires the production of fertile F_1 progeny, so we will confine the argument to this. In the case of *B. tabaci*, how often fertile heterozygotes are produced between the many different biotypes is unclear. Lui *et al*. (1992), Costa *et al*. (1993) and Bedford *et al*. (1994) failed to demonstrate interbreeding of the B biotype and the USA A biotype, whereas Byrne *et al*. (1995) succeeded in producing hybrid females between the B biotype and its closest relative, the Sudanese L biotype. It was not stated if these were fertile. Gunning *et al*. (1997) found

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hybrids between the indigenous non-silverleafing *B. tabaci* from northwestern Australia and the introduced B biotype in field collected material. Again, it was not stated whether or not the hybrids were fertile. Hybrids between the B biotype and the indigenous *B. tabaci* from eastern Australia have also been found in samples of *B. tabaci* collected in southeastern Queensland as part of an ongoing B biotype surveillance programme (P. De Barro, unpublished). Whether these were fertile remains unknown.

On the basis of the differences between the B biotype and the A biotype, Bellows *et al*. (1994) concluded that the *B. tabaci* B biotype was a different species and revised the name to *B. argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae). This is contentious given that the B complex of biotypes is concentrated in the Mediterranean/north east Africa/Middle East region, from which the original syntypes were collected by Gennadius in 1889 (Brown *et al*., 1995a; Frohlich *et al*., 1999; De Barro *et al*., in press). Hence, we have chosen to retain the original biotype designation *B. tabaci* biotype B.

The ability to interbreed successfully is not just a taxonomic issue. There may be biological consequences that affect the likelihood of a successful incursion by an exotic biotype and/or understanding of the field dynamics which may greatly affect pest phenology and disease epidemiology. Phylogenetic studies have revealed the presence of distinct geographical boundaries separating populations of different biotypes, i.e. Mediterranean, north east Africa, Middle East, Turkey and Pakistan and eastern and western populations of the indigenous Australian biotype of *B. tabaci* (De Barro *et al*., in press). Further, field collections in Australia have shown the exotic B biotype to be uncommon in parts of Queensland and New South Wales (where the indigenous Australian *B. tabaci* is common) despite the reverse being true in the surrounding areas. In the latter case, the distributions could not be explained adequately by differences of climate, plant host availability or natural enemy biodiversity as these were similar across both regions. Therefore, it was hypothesized that some form of mating isolation between the different biotypes was responsible for the observed patterns of distribution. Both pre-mating and post-mating isolation are well known among geographic races, subspecies and sibling species of a number of arthropods including mites (Caprio & Hoy, 1995), Coleoptera (Majerus *et al*., 1986), Diptera (Grimaldi *et al*., 1992), Hymenoptera (Breeuwer *et al*., 1992; Spollen & Hoy, 1992) and Lepidoptera (Byers & Hinks, 1978). The clearest example of reproductive incompatibility between different biotypes of *B. tabaci* is the displacement of the American A biotype by the B biotype (Perring *et al*., 1994). In the present Australian context, a series of experiments were undertaken to determine whether the relative scarcity of the B biotype in some parts of the continent was indeed a consequence of mating isolation between biotypes.

Materials and methods

Whitefly cultures and adult collection

The exotic B biotype and indigenous eastern (EAN) and western (WAN) Australian *B. tabaci* populations were maintained in separate cultures on *Euphorbia cyathophora* Murray (Euphorbiaceae) in separate constant temperature rooms (12:12 L/D, 22 ± 1°C). Preliminary studies showed that the relative longevity and fecundity of EAN and B biotypes on this host were not significantly different, but both populations significantly outperformed the WAN population. Cultures were initially established using several thousand field-collected adults. The cultures were maintained for two years in Canberra with annual additions of large numbers of field collected individuals to reduce the likelihood of inbreeding. The cultures were regularly tested for purity using RAPD-PCR (random amplified polymorphic DNA polymerase chain reaction). The protocol is described in De Barro & Driver (1997), but briefly, individual whiteflies were homogenized in 25 μl of lysis buffer (50 mM KCl, 10 mM Tris pH 8.4, 0.45% Tween 20, 0.2% gelatin, 0.45% NP40, 60 μg ml^{-1} proteinase K). The homogenate was incubated at 65°C for 30 min and then boiled for 10 min. Sterile distilled water (25 μl) was then added to yield a final homogenate volume of 50 μl. The PCR conditions were as follows.

All reaction volumes were 25 μL, containing 0.3 μM of primer (H16 and F5; Operon Technologies, INC, California, USA), 200 μM each dGTP, dATP dCTP and dTTP, 3.5 mM $MgCl_2$, 1 μl DNA lysate, 1X supplied buffer and 2.5U Taq polymerase (Fisher Biotech, Australia). PCR amplifications were done in a Hybaid thermocycler. One cycle of 94°C for 5 min, 40°C for 2 min and 72°C for 3 min followed by 39 cycles of 94°C for 1 min, 40°C for 1.5 min and 72°C for 2 min. Amplification products were separated electrophoretically using 1.5% agarose run at 8.0 V/cm for 2 h. A 100bp molecular weight marker (Pharmacia Biotech, 27-4001-01) was included in each gel. Bands were made visible using UV light.

Whiteflies used in the experiments were collected from cultures as fourth instar red eye pupae and placed in emergence cages. As *B. tabaci* adults <12 h old do not mate (Li *et al*., 1989), only those that had emerged within this period were used to ensure their virginity. For field cage experiments, 120 adults were collected into glass vials, together with additional samples to assess starting sex ratios and confirm biotype purity. Males and females from each biotype were collected separately into glass vials for fertility/fecundity and hybridization experiments. In each case, adults were kept cool (4°C) to prevent dehydration.

Comparison of rates of development between the B, EAN and WAN biotypes

Single mated females of the B, EAN or WAN biotypes were allowed to oviposit for 24 h onto *E. cyathophora* seedlings kept in cylinder cages at 15°C, 20°C, 25°C or 30°C. Each treatment was replicated ten times per temperature. After 24 h the insects were removed. The time taken for development from newly laid egg to emerging adult was noted. Linear regression was then used to determine whether temperature had a significant influence on development and lines were compared to determine whether rates of development were significantly different between the three biotypes (Zar, 1984).

Field population experiments between pure and mixed B and EAN biotype populations

Field cage experiment 1

In order to study interaction between exotic and indigenous *B. tabaci* biotypes, experiments were performed to compare single biotype populations with mixed populations. Six, 2 m^3 fine gauze covered cages were set out

in a single replicated 2 × 3 block. Into each cage, six *E. cyathophora* plants in 20 cm diameter pots were buried in the soil, and watered using an automated drip irrigation system. The soil was treated with herbicide and then covered with a thick layer of mulch to discourage weed growth and provide a good seal between cage and ground surface. During the experiment, weeds that emerged were removed. Plants were fertilized regularly using 1 teaspoon of the slow release fertilizer, Osmocote®. Predatory mites, *Phytoseiulus persimilis* Athias-Henriot (Acari: Tetranychidae) were added each month to prevent infestation with two-spotted mites, *Tetranychus urticae* Koch (Acari: Tetranychidae). There were three treatments in all; B alone, EAN alone and a 50:50 mix of the two biotypes, each replicated twice.

Into each cage, 20 whiteflies were added per plant in a ratio later determined as 1:2 (male:female). Cages were then left undisturbed until completion of development of the first generation. At this time, one branch was collected from each plant per cage. *Euphorbia cyathophora* produces branches that originate from the base and at the time of sampling, plants had between five and six branches. On each branch, leaves range from the oldest to the youngest and as such, represent a good sample of the whitefly population present. The branches from each plant were kept separate and the leaves were then removed, the number of eggs, juveniles and emerged pupae counted and leaf area measured using a Li-Cor LI-3000A area meter. Numbers of eggs and nymphs were transformed to per cm^2 of leaf. The sex ratio for each sample was also determined by examining 60 adults (ten per plant) collected at random per cage. Taking female progeny only, 47 from each of the single biotype cages and 150 from each of the mixed biotype cages were analysed using RAPD-PCR to determine: (i) whether contamination of the single biotype cages had occurred; and (ii) the biotype ratio in the mixed biotype treatments. A second sample was taken when the next generation was completed. Owing to a large increase in whitefly numbers, ten leaves from each of the six plants in each cage were sampled at random from across each plant. Leaves were then processed and whitefly numbers, sex ratio and biotype determined as before.

Within cage variation of egg and nymph densities between each of the six branches was tested using ANOVA. There was no difference in densities between branches so the counts per branch per cage were pooled. Data were analysed using two way ANOVA with biotype mix, cage and the interaction tested.

Field cage experiment 2

This experiment repeated the methodology of the first experiment, except that it was run for three generations, ten whiteflies per plant were added and, in the first sample, ten leaves per plant per cage were collected. The experiment was extended a further generation due to a suggested change in the sex ratio at the end of the second generation in the first field cage experiment while the number of whiteflies added was reduced so that the plants would survive.

Fertility/fecundity of B and EAN biotypes

Fertility/fecundity experiment 1

To determine the cause for the reduced population growth observed in field cages, pairs of unmated whiteflies were caged in 50 × 22 mm glass vials stoppered at either end with a plastic plug. One was filled with 1.3% agar onto which a disk of *E. cyathophora* (which completely covered the agar) was placed leaving the underside of the leaf exposed. The vial was left upright with the stopper containing the leaf disk at the top. Whiteflies were able to feed and oviposit onto the leaf disks. They were observed daily and, whenever a male died, a new male of the same biotype was introduced. Courtship was also noted. Any dead males were placed into 95% ethanol for later biotype determination. Leaf disks were replaced with new disks on new agar every five days and the number of eggs deposited onto each of the disks, counted. Tubes were also replaced as they became fouled with honeydew. After counting, disks were dipped in a fungicide solution (0.375 g zineb + 3.5 ml triforine in 250 ml dH_2O) and allowed to dry before being left for ten days on damp, fungicide treated filter paper in individual petri dishes. After this time, the number of eggs hatched on each disk was counted. Once the female died, the particular replicate was considered complete and the female and remaining male were placed in ethanol. The experiment was terminated after 35 days. There were four treatments replicated 12 times: B male + B female, EAN male + EAN female, B male + EAN female and EAN male + B female. The experiment was run in a constant temperature room (12:12 L/D, 22 ± 1°C). Data were analysed using one way ANOVA followed by Tukey test of multiple comparisons. Biotype identity of adults was confirmed using RAPD-PCR.

Fertility/fecundity experiment 2

As male whiteflies are produced from unfertilized eggs, it was possible that differences observed between treatments in the first fertility/fecundity experiment may have been due to a lack of mating. This experiment was therefore repeated with the addition of two more treatments, unmated B female only and unmated EAN female only, with a reduction in the number of replicates to ten per treatment.

Detection of hybrids

Ten newly emerged female and five male whiteflies were placed in cylinder cages (15 × 30 cm) on *E. cyathophora* growing in 15 cm pots. There were nine treatments: B females + B males, EAN females + EAN males, WAN females + WAN males, B females + EAN males, B females + WAN males, EAN females + B males, EAN females + WAN males, WAN females + B males and WAN females + EAN males, each replicated five times. After adding whiteflies, tape was used to seal the cage to the pot to prevent contamination and the experiment was then left to run for 18 days in a glasshouse (T°C ranged from 25° to 30°C). Thereafter, all adults, dead or alive, were removed. Plants were again caged and left until the F_1 generation adults began to emerge. These were put into ethanol in a 5°C cool room for sex ratio determination at a later date.

The identity of 23 females and 23 males from each biotype cross and ≥80 females and 20 males from each of the mixed crosses were determined using RAPD-PCR. The abdomens were removed from F_1 females from mixed crosses to ensure that DNA amplified was not contaminated with sperm. Prior to determination of the biotype, ten females and ten males from each of the single biotype crosses and a minimum of five females and 20 males from each of the hybrid crosses

were dissected into insect ringer solution. The dissected abdomens were then mounted on slides and viewed with the aid of a phase contrast compound microscope (200×) to determine whether the individual was fertile. Individuals were presumed fertile if their abdomens were normal in appearance and contained ovaries or testes that did not appear different to those from individuals from the cultures.

Results

Comparison of rates of development between the B, EAN and WAN biotypes

The number of days to complete development is summarized by the following equations:

B biotype = -0.58Temperature + 35.85, $r^2 = 0.96$
EAN = -0.61Temperature + 39.1, $r^2 = 0.96$
WAN = -0.56Temperature + 39.45, $r^2 = 0.93$.

In each case, the time taken to complete development decreased linearly ($P < 0.0001$, fig. 1) with increasing temperature. There was no difference in the slopes of the three lines indicating they were parallel; however the intercepts for the three lines differed significantly ($P < 0.01$). The B biotype completed development two days faster than the EAN which in turn completed development one day faster than the WAN biotype.

Field population experiments between pure and mixed B and EAN biotype populations

Within each cage there was no difference in mean numbers of whitefly eggs and nymphs per cm^2. The mean numbers of eggs and nymphs per cm^2 produced in the cages containing single biotypes were significantly greater (generation 1, $P < 0.0001$; generation 2, $P < 0.0001$) than the mean numbers produced in cages containing mixed biotypes in both generations in 'Field cage experiment 1' and the first two generations in 'Field cage experiment 2'. In the third generation of 'Field cage experiment 2', numbers across all treatments were not significantly different (generation 1, $P < 0.0001$; generation 2, $P < 0.0001$, generation 3, $P > 0.05$; fig. 2a and b).

The 1:2 ratio of males to females remained constant for the single biotype treatments throughout both experiments. However, the ratio in the mixed biotype treatment switched to male dominance for the first two generations in both experiments before returning to female dominance in the third generation (fig. 3a and b). The difference between the proportion of females in mixed biotype and single biotype treatments was significant for each generation ('Field cage experiment 1' generation 1, $P < 0.001$, generation 2, $P < 0.01$; 'Field cage experiment 2' generation 1, $P < 0.0001$, generation 2, $P < 0.001$, generation 3, $P < 0.05$).

The proportion of B biotype individuals in cages started with equal numbers of both biotypes increased from 0.50 to ~0.66 over the two generations of 'Field cage experiment 1' (table 1). This increase was repeated in 'Field cage experiment 2' where after three generations, the proportion was ~0.84 (table 1). In each cage, a small proportion of the females produced were hybrids (table 1).

Fertility/fecundity of B and EAN biotypes

There were no significant differences between replicates within each treatment in either of the experiments. There was no significant difference between treatments in average

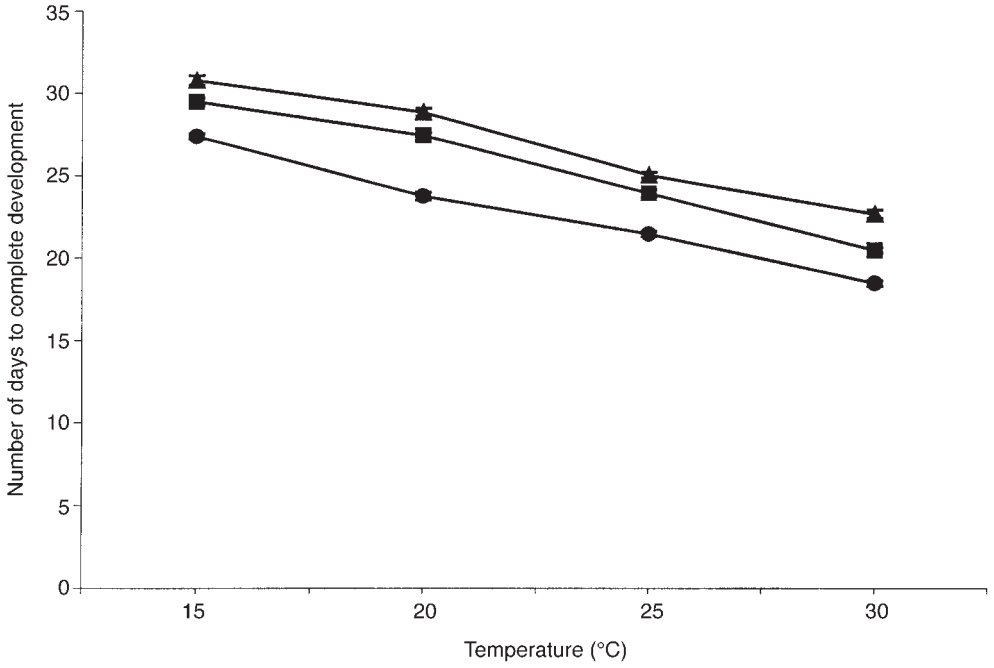

Fig. 1. The rate of development of *Bemisia tabaci* biotype B (●), and the indigenous EAN (■) and WAN (▲) biotypes.

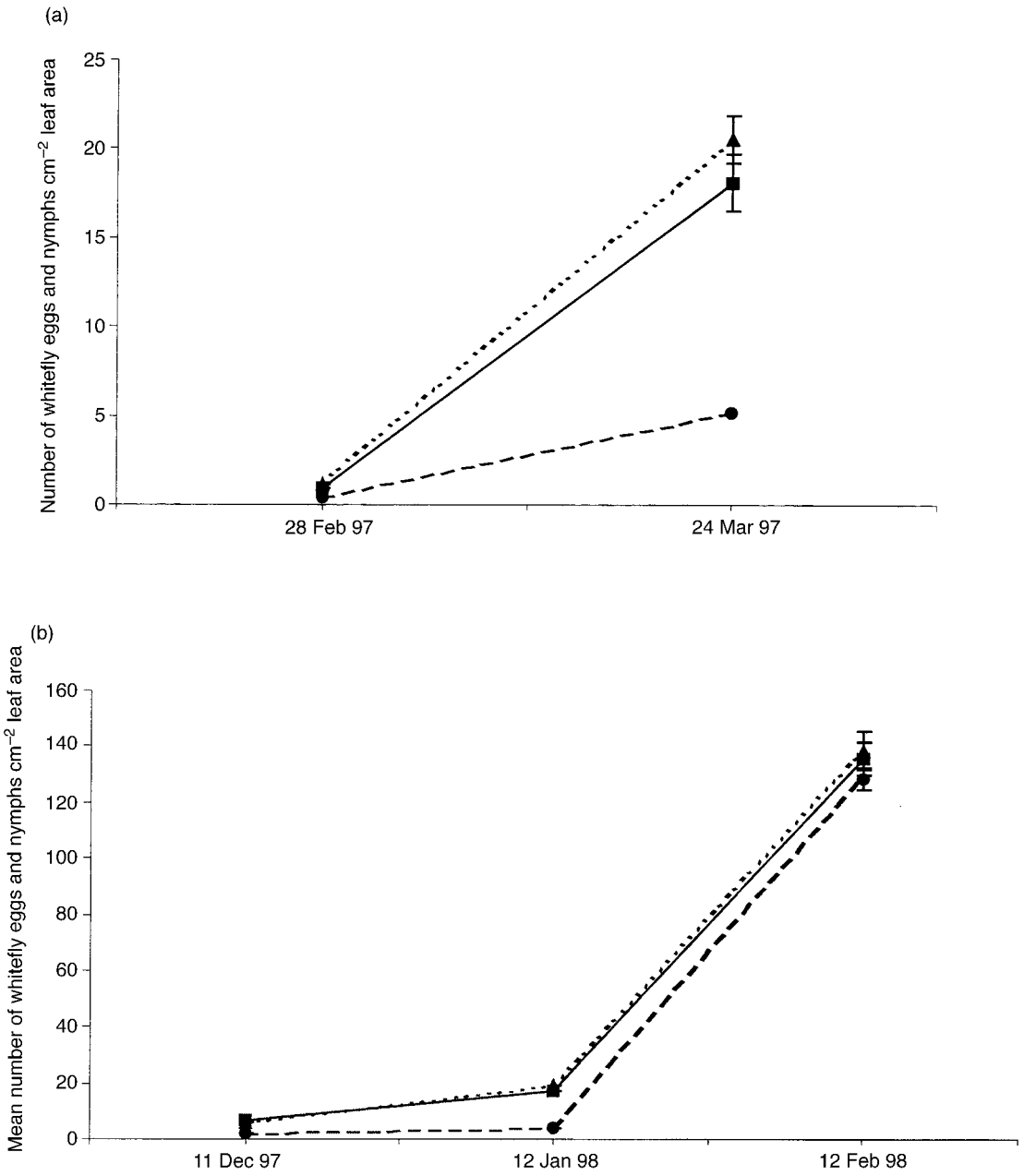

Fig. 2. Mean number of eggs and nymphs cm^{-2} of leaf in single biotype (B and EAN biotype) and mixed biotype (B+EAN) cages containing whiteflies over (a) two or (b) three generations. The different treatments are indicated by B biotype (■), EAN biotype (▲) and mixed biotype (●).

female survival or in the mean number of eggs hatched in either experiment (table 2). Male and female whitefly were observed to form courting pairs in all treatments and replicates with mixed biotype pairs spent more time as courting pairs than did single biotype pairs (table 2). The females in Experiment 1 from both of the single biotype crosses had an average egg production of approximately ten eggs per day. This was significantly higher ($P < 0.001$; table 2) than for the mixed biotype. The direction of the cross had no significant effect. In the second experiment, there were no significant differences between the average numbers of eggs laid per day by unmated females of either biotype and females paired with males of the same biotype (table 2). As with the first experiment, a female in the presence of a male of a different biotype laid significantly fewer eggs ($P < 0.0001$) and spent more time as a courting pair (table 2).

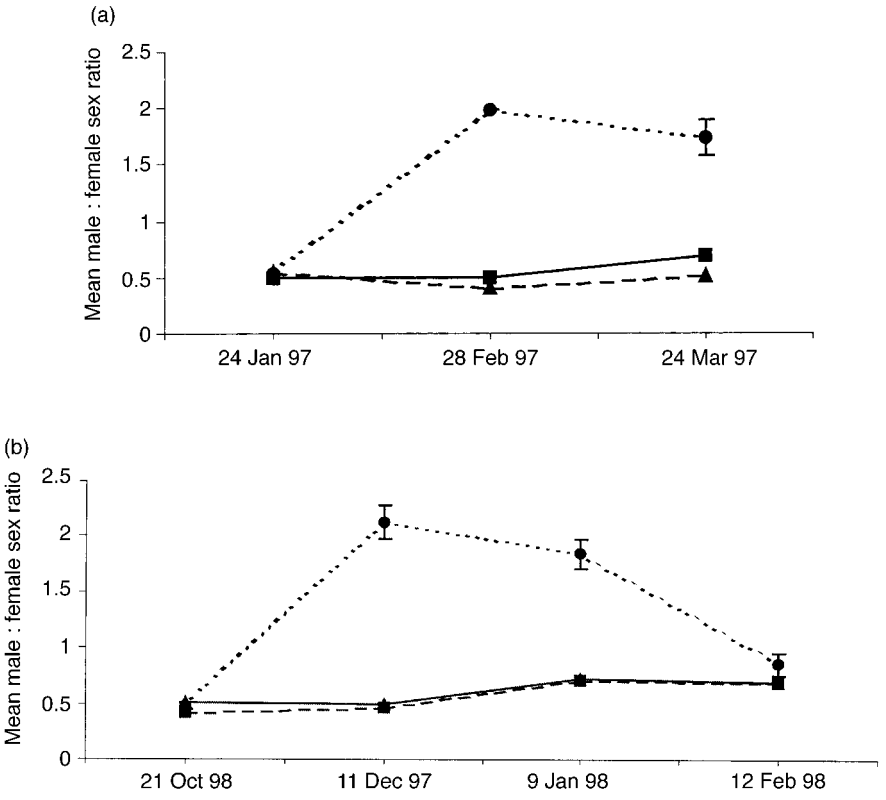

Fig. 3. Mean male:female sex ratio in single biotype (B and EAN biotype) and mixed biotype (B+EAN) cages containing whiteflies over (a) two or (b) three generations. The different treatments are indicated by B biotype (■), EAN biotype (▲) and mixed biotype (●).

Detection of hybrids

In the experiment to detect hybrids, there were no significant differences between replicates within treatments. There was a significant difference in the proportion of F_1 females produced in the various crosses ($P < 0.0001$; table 3). Single biotype matings produced a greater proportion of F_1 females ($P < 0.001$) than mixed biotype crosses which produced very few females. Of the mixed biotype crosses, those crosses between EAN and WAN individuals produced a greater proportion of F_1 females than crossing B and WAN biotypes. Both sets of crosses produced a greater proportion of females than crosses between B and EAN (table 3). All females produced in the mixed biotype crosses were hybrids as they showed RAPD-PCR profiles characteristic of both biotypes (fig. 4a and b). In addition, all F_1 hybrid females from both EAN/B and WAN/B crosses had shrunken abdomens with non-functional ovaries. In contrast, F_1 females from same biotype crosses had normal plump abdomens containing functional ovaries. The results from the EAN/WAN crosses had elements from both the above outcomes producing F_1 female progeny that were presumed either sterile (EANf × WANm, n = 69; WANf × EANm, n = 76) or fertile (EANf × WANm, n = 11; WANf × EANm, n = 4). F_1 males from all crosses appeared normal and had RAPD-PCR profiles that reflected the maternal profile. Upon dissection, there were no differences between the testes of F_1 males derived from any of the crosses and they were presumed fertile.

Discussion

This study supports the view that some form of mating isolation is operating, at least partially, between the different biotypes of *B. tabaci*. The results confirm the observation of Byrne *et al.* (1995) that hybridization occurs between the B biotype and non-B biotypes, but goes further in indicating that these hybrids may be sterile and certainly are in the B biotype–Australian biotypes matings. The key results of the interaction were reduced population increase, a marked increase in the proportion of male progeny, fewer eggs produced by females paired with males of different biotype and no difference in the numbers of eggs per unmated female and females paired with males of the same biotype. In addition, there was no change in the proportion of eggs hatching; mixed biotype pairs spent more time courting than single biotype pairs and a low level of hybridization in field cages and small containers was observed. Both pre-mating and post-mating isolation leading to a failure of fertilization would explain these observations. However, the mechanisms driving such isolation are less obvious.

Three mechanisms provide possible explanations. The first is an example of pre-mating isolation and relies on the observation that female whitefly do not lay eggs when courting. The experiment on fecundity demonstrated that males and females of different biotypes were unable to recognize the difference and entered into courtship, as also observed between the B and A biotypes by Bellows *et al.*

Table 1. Proportion of B biotype and hybrid individuals in cages containing a mixture of both B and EAN biotypes over two (Field cage experiment 1) or three (Field cage experiment 2) generations.

		Mean proportion of B biotype whitefly in mixed populations cages		Mean proportion of hybrid whitefly in mixed populations cages	
	Generation	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Field cage experiment 1					
24 Jan 97	Setup	0.50	0.50	0	0
28 Feb 97	1	0.55	0.57	0.02	0.01
24 Mar 97	2	0.65	0.67	0.04	0.03
Field cage experiment 2					
21 Oct 97	Setup	0.50	0.50	0	0
11 Dec 97	1	0.49	0.53	0.01	0.01
9 Jan 98	2	0.64	0.62	0.03	0.05
12 Feb 98	3	0.83	0.84	0.04	0.02

Table 2. The number of days survived, the number of eggs laid and hatched and the time spent courting for matings in same and different biotype crosses of *Bemisia tabaci*.

Treatment (F × M)	Number of days female survival	Number of eggs per day	Proportion of eggs hatched	Proportion of time courting
Fertility/fecundity experiment 1				
B × B	23.4±2.9	10.1±1.17[a]	0.68±0.03	0.41±0.05[a]
EAN × EAN	26.5±2.5	10.2±0.6[a]	0.67±0.06	0.32±0.08[a]
B × EAN	24.3±2.8	5.8±0.4[b]	0.68±0.04	0.64±0.09[b]
EAN × B	25.3±2.5	7.0±0.4[b]	0.72±0.03	0.72±0.06[b]
	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P < 0.05$
Fertility/fecundity experiment 2				
B × B	26.7±2.63	10.56±1.48[a]	0.62±0.02	0.39±0.08[a]
EAN × EAN	25.3±4.0	10.31±0.51[a]	0.71±0.03	0.44±0.04[a]
B × EAN	30.7±2.38	5.83±0.89[b]	0.62±0.06	0.72±0.11[b]
EAN × B	24.7±1.00	4.04±0.55[b]	0.57±0.03	0.68±0.05[b]
B female only	27.6±3.46	9.61±0.5[a]	0.63±0.03	–
EAN female only	31.9±1.74	9.31±0.47[a]	0.66±0.03	–
	$P > 0.05$	$P < 0.0001$	$P > 0.05$	$P < 0.05$

All means are ± standard error. Superscripts indicate whether means were significantly different.

(1994). However, this presumes that the pair had a greatly reduced ability to complete courtship with successful copulation and so continue to devote time to courtship. The result is that females have less time to lay eggs than those that have completed copulation or virgin females in the absence of a male. This suggests the presence of an incompatible male distracts the female from egg laying and when she does lay eggs, they are likely to be unfertilized and hence develop into males. We have called this the 'distracting male hypothesis'. The low proportion of hybrids produced indicates copulation leading to successful fertilization does take place, but equally the low proportion of hybrids indicates a high degree of incompatibility between biotypes.

Table 3. Mean proportion ± standard error of F_1 female whitefly progeny resulting from crosses between the three different biotypes, B, EAN and WAN.

Cross	Proportion of female F_1 progeny
B♀ × B♂	0.61 ± 0.02[a]
EAN♀ × EAN♂	0.69 ± 0.02[a]
WAN♀ × WAN♂	0.62 ± 0.01[a]
B♀ × EAN♂	0.06 ± 0.02[d]
B♀ × WAN♂	0.15 ± 0.005[c]
EAN♀ × B♂	0.007± 0.005[d]
EAN♀ × WAN♂	0.30 ± 0.02[b]
WAN♀ × B♂	0.17 ± 0.02[c]
WAN♀ × EAN♂	0.30 ± 0.02[b]

Superscripts indicate whether means were significantly different.

The second mechanism draws upon the single-locus complementary sex determination (CSD) model developed for haplo-diploid species (Whiting, 1943; Cook, 1993; Cook & Crozier, 1995). The model has been extensively tested in the Hymenoptera, but not to species outside this order. Under the single-locus CSD model, sex is determined by multiple alleles at a single locus. At this locus, heterozygotes (diploid) are female, hemizygotes (haploid) male and homozygotes (diploid) also male, but not usually viable. In such a system of post-mating isolation, as occurs in *Apis mellifera* Linnaeus (Hymenoptera: Apidae), inbreeding of colonies leads to increased homozygosity and a resultant increase in diploid males at the expense of heterozygous females resulting in an overall increase in the proportion of males and a decline in colony size (Cook, 1993).

Our observations showed a significant decline in egg production in mixed matings relative to unmated and same biotype matings. We also know successful fertilization occurs as some hybrid female progeny are produced. So, the observed decline in fecundity in crosses between different biotypes could be due to successful copulations leading to the production of diploid male eggs that die during

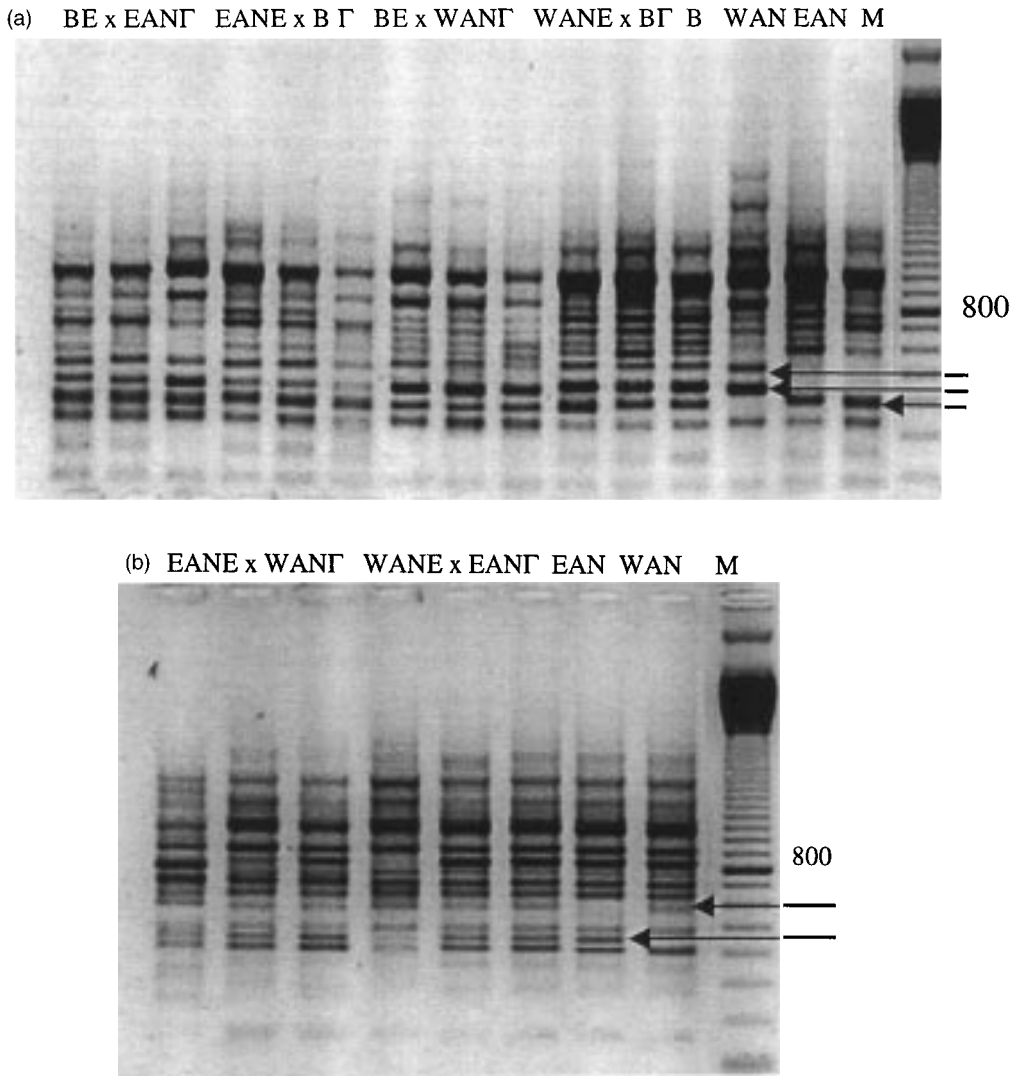

Fig. 4. RAPD PCR profile of F_1 female progeny from crosses between (a) B biotype, and EAN and WAN biotype individuals using primer H16 or (b) EAN and WAN biotype individuals using primer F5. M indicates the 100 base pair ladder with the 800 base pair band indicated. B, EAN and WAN are the standard profiles of the three biotypes using (a) primer H16 or (b) primer F5. Informative bands are marked with an arrow.

embryogenesis and are resorbed by the female. This would be a departure from what is normally seen with Hymenoptera and is not known to occur with whiteflies. This would explain the failure to detect hybrid males and the observed similarity in egg hatch whilst increase in the proportion of males and the slower rate of population increase could result from the production of fewer female progeny at the expense of more diploid males.

However, for this model to be valid it would need one of the two biotypes to have fewer alleles and to share a large number of these with the other biotype, otherwise there would be no increase in the proportion of diploid males relative to the single biotype crosses. This is possible since the B biotype is a recent incursion to Australia and as with most incursions, probably involved a small number of individuals that in turn were likely to possess a small subset of the possible sex determination alleles (e.g. *Solenopsis invicta* Buren (Formicidea), Ross *et al*., 1993).

The third possible cause for the observed interaction is cytoplasmic incompatibility as a result of infections by rickettsias belonging to the genus *Wolbachia*. These endosymbionts are known to cause complete or partial post-mating reproductive isolation between species and populations e.g. *Nasonia vitripennis* (Walker) and *N. giraulti* Darling (Hymenoptera: Pteromalidae) (Bordenstein & Werren, 1998), *Ephestia cautella* (Walker) and *E. kuehniella* Zeller (Lepidoptera: Pyralidae) (Sasaki & Ishikawa, 1999) and populations of *Drosphila melanogaster* (Meigen) (Diptera: Drosophilidae) (Hoffman, 1988). Mating between incompatible individuals in haplo-diploid species leads to male production. If cytoplasmic incompatibility is occurring here it is bidirectional, as both the cross and its reciprocal leads to predominantly male production, but incomplete as some female hybrid progeny are produced. However, as these are infertile it suggests, as in the case of *Encarsia formosa* Gahan (Zchori-Fein *et al*., 1992; Werren, 1997), the situation

has persisted for a sufficient period of time to allow the accumulation of enough mutations in the genes governing sexuality to prevent the production of fertile hybrids.

The proportion of B biotype individuals in the mixed cages increased over three generations to a point where they dominated the population and the male to female ratio returned to that observed in the single biotype populations. Given that females from both biotypes have similar longevities and fecundities, the most likely explanation lies in the comparison of the rates of development of the B and EAN biotypes. Here, the B biotype completed its development approximately two days faster than the EAN biotype. This means that at certain times there would be a large increase in B biotype adults relative to EAN adults increasing the chance of same biotype matings. This lack of synchrony was predicted by Sutherst (1987) to result in an effective increase in relative frequency and so favour establishment.

This and the outcomes from between-biotype interactions suggests there is a threshold operating that requires a minimum number of B biotype adults relative to EAN biotype adults to be present in order for establishment to occur. This may explain the observed rarity of the B biotype in regions where EAN is numerically dominant. That is, in situations where the EAN biotype is abundant, invading B biotype individuals are likely to be at a numerical disadvantage. Therefore, both female and male B biotypes, given their inability to distinguish between biotypes, are more likely to encounter and attempt to mate with individuals from the EAN biotype. This will lead to some or all of the following: fewer eggs being laid, no fertile female progeny and a slow rate of population growth. Together, these are likely to have a significant negative impact on the likelihood of establishment. The situation is likely to remain so until some change in the environment favours establishment. An establishment threshold may also explain the strong biogeographic structure of the distributions of several biotypes from across the North Africa, Middle East, Mediterranean and western Asia regions. Here, both Frohlich *et al*. (1999) and De Barro *et al*. (in press) found virtually no overlap in the distributions of several different biotypes, with the possible exception of the B biotype and its sibling, the Sudanese L biotype (Bedford *et al*., 1994).

Results from crosses between EAN and WAN individuals are also interesting in that they show how the *B. tabaci* complex may have arisen through geographic isolation. The two populations are separated by a mountain range and a fairly arid region to the west of the range. Biologically, the two populations are distinct with the EAN biotype having a broader host range and faster rate of development than the WAN biotype. The high proportion of males and large proportion of sterile hybrid females point to there being a high degree of genetic incompatibility, but that the barrier is not yet complete.

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